Electronic Supplementary Material (ESI) for Organic Chemistry Frontiers.

Supporting Information

for

Synthesis of Bisarylethyne-Peptide Conjugates

by

M. Strack, S. Langklotz, J. E. Bandow, N. Metzler-Nolte, H. B. Albada*

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General Information

All solution-phase modifications were performed using oven-dried glassware under a nitrogen atmosphere and standard Schlenk techniques. Peptide chain assembly was performed in plastic syringes with a porous polypropylene disc as filter. Chemicals were obtained from commercial suppliers and used without further purification; solvents were dried according to standard procedures and stored over molecular sieves (3 Å).

Preparative HPLC was performed on an HPLC system with PDA detector. For this, a HIBAR Lichrospher 100 RP-18e reversed phase column (250 × 25 mm) was used at a flow rate of 20 mL/min. Linear gradients of 5% B/min starting with 5 min of buffer A were used (A: H_2O/MeCN/TFA 95:5:0.1 v/v/v; B: MeCN/H_2O/TFA 95:5:0.1 v/v/v).

NMR spectroscopy was performed in deuterated solvents at 30 °C on a Bruker DPX-200, DPX-250 or DPX-400 device. Chemical shifts (δ) are given in parts per million (ppm) relative to TMS and the solvent residual signal is used as a reference. Abbreviations for peak multiplicities are s (singlet), d (doublet), t (triplet), m (multiplet) and br (broad). Some ^{13}C chemical shifts were extracted from 2D-H,C-correlation spectra.

Mass spectrometry was performed on a Bruker Esquire 6000 mass spectrometer (ESI) or a VG Instruments Autospec mass spectrometer (EI). The *m/z* ratio is given as a dimensionless number.

High-resolution mass spectrometry data were acquired using a Synapt G2-S HDMS instrument (Waters) equipped with a lock spray source for electrospray ionization (ESI) and a time of flight (ToF) detector. For an analysis, samples were diluted 1:10 in 50% acetonitrile/0.1% formic acid and were injected for direct infusion with a flow of 0.5 µL/min. Spectra were recorded in positive ionization mode over a mass range of 50 to 1200 *m*/*z* with 1 s/scan. The following parameters were used for the NanoLockSpray source: capillary voltage, 3.0 kV; sampling cone voltage, 30 V; source temperature, 100°C; desolvation temperature, 150°C; cone gas flow; 50 L/h; desolvation gas flow, 500 L/h. Leucine enkephaline serving as lock mass analyte was fed through the lock spray channel (lock mass capillary voltage, 3.5 kV). Analysis of the spectra was performed using MassLynx V4.1 SCN883 (Waters).

Synthesis

Phenylacetylene-derivatized Peptides

Synthesis of Ac-[4-(2-phenylethynyl)-Phe]-Xaa-Rink, (Xaa = Ala (1), Ser(^{t}Bu))

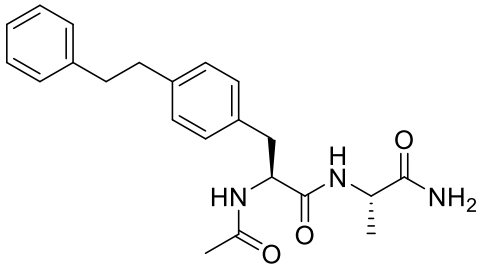
Peptide synthesis was performed as follows: A solution of Fmoc-AA-OH (4 eq.), HOBt (4 eq.) and TBTU (3.8 eq.) in DMF (7 mL) was prepared, and D*i*PEA (8 eq.) was added shortly before addition to a Rink amide resin (1 g, 0.71 mmol/g, 1 eq.). The suspension was agitated for 1 h at room temperature. The reaction mixture was removed by filtration, and the resin was washed with DMF (4 × 7 mL × 2 min) and DCM (3 × 7 mL × 2 min). Fmoc deprotection was accomplished by two consecutive treatments with 20 % piperidine in NMP (2 × 7 mL × 10 min) and subsequent washing of the resin with DMF (4 × 7 mL × 2 min) and DCM (3 × 7 mL × 2 min). After final Fmoc deprotection, the N-terminus was acetylated with a solution of Ac_2O (0.5 M) and D*i*PEA (0.125 M) in NMP (2 × 7 mL × 10 min), and the solid support was

washed with DMF (4 × 7 mL × 2 min), DCM (3 × 7 mL × 2 min), Et_2O (3 × 7 mL × 2 min) and was dried *in vacuo*. For the Sonogashira cross-coupling, dry peptide-bound resin was placed in an oven-dried Schlenk flask and DMF (7 mL), D*i*PEA (482.1 µL, 2.84 mmol), and phenylacetylene (156 µL, 1.42 mmol) were added. The mixture was degassed with N_2 for 15 min, and $(PPh_3)_2PdCl_2$ (249.2 mg, 0.36 mmol) and CuI (135.2 mg, 0.71 mmol) were added. The suspension was stirred at room temperature overnight. The resin was isolated by filtration and washed with DCM (5 × 10 mL × 2 min), DMF (5 × 10 mL × 2 min), DCM (4 × 10 mL × 2 min) and Et_2O (3 × 10 mL × 2 min) and was dried *in vacuo*.

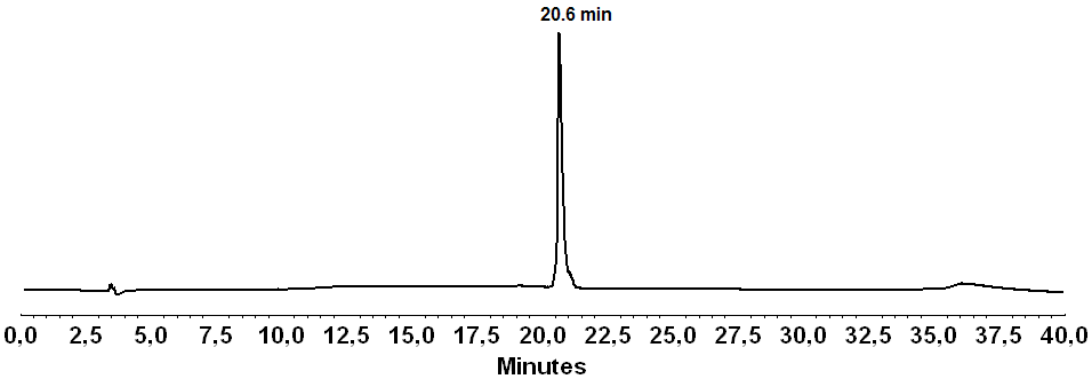
General Procedure A: reduction of the triple bond using hydrogen or deuterium containing cleavage reagents

A solution of hydrogen or deuterium containing TFA/H_2O/TES (80:10:10, v/v/v, V_{tot} = 1.5 mL) was added to dry Ac-[4-(2-phenylethynyl)-Phe]-Ala-Rink resin (0.15 g, 81.1 µmol). The mixture was agitated over night at room temperature, the solution was separated by filtration and the resin was washed with TFA (2 × 1 mL × 1 min). All TFA fractions were combined and concentrated *in vacuo*. Addition of ice-cold Et_2O (25 mL) followed by centrifugation and two washing cycles with Et_2O (25 mL) yielded the crude materials.

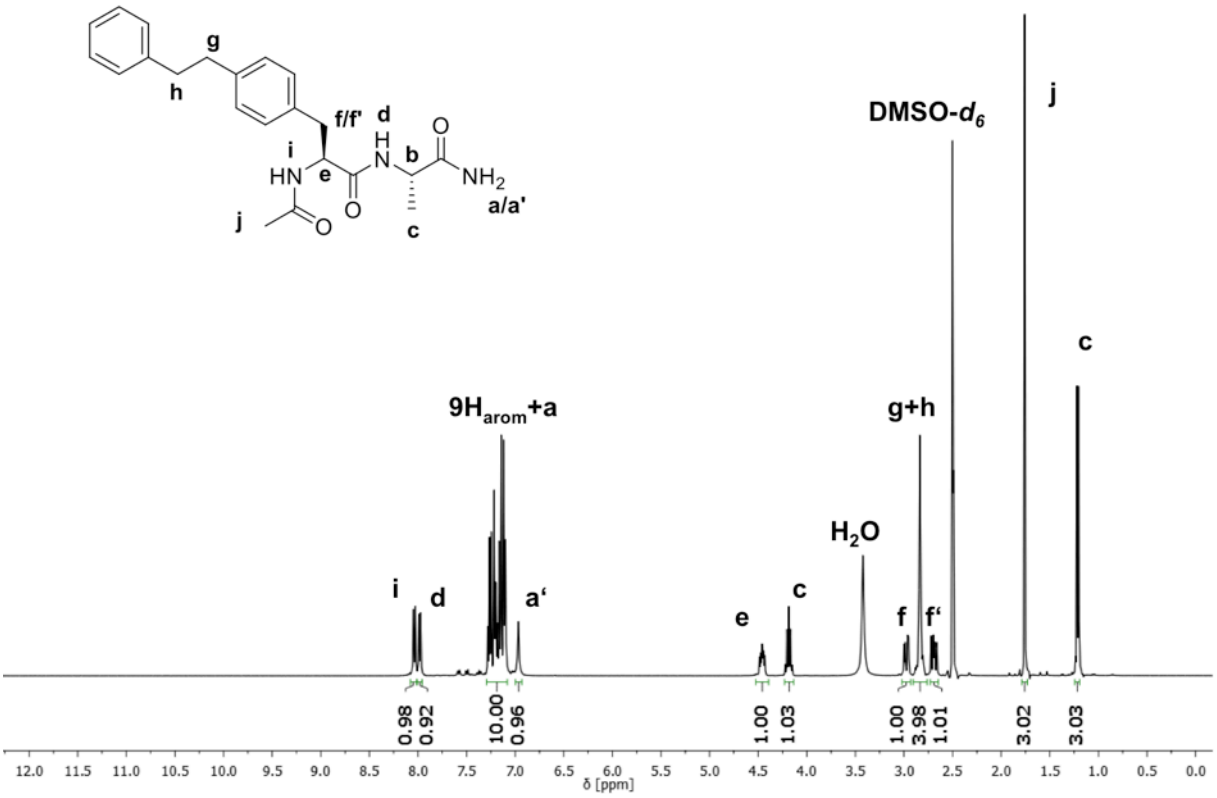
Ac-[4-(2-phenylethyl)-Phe]-Ala-NH_2 (4)



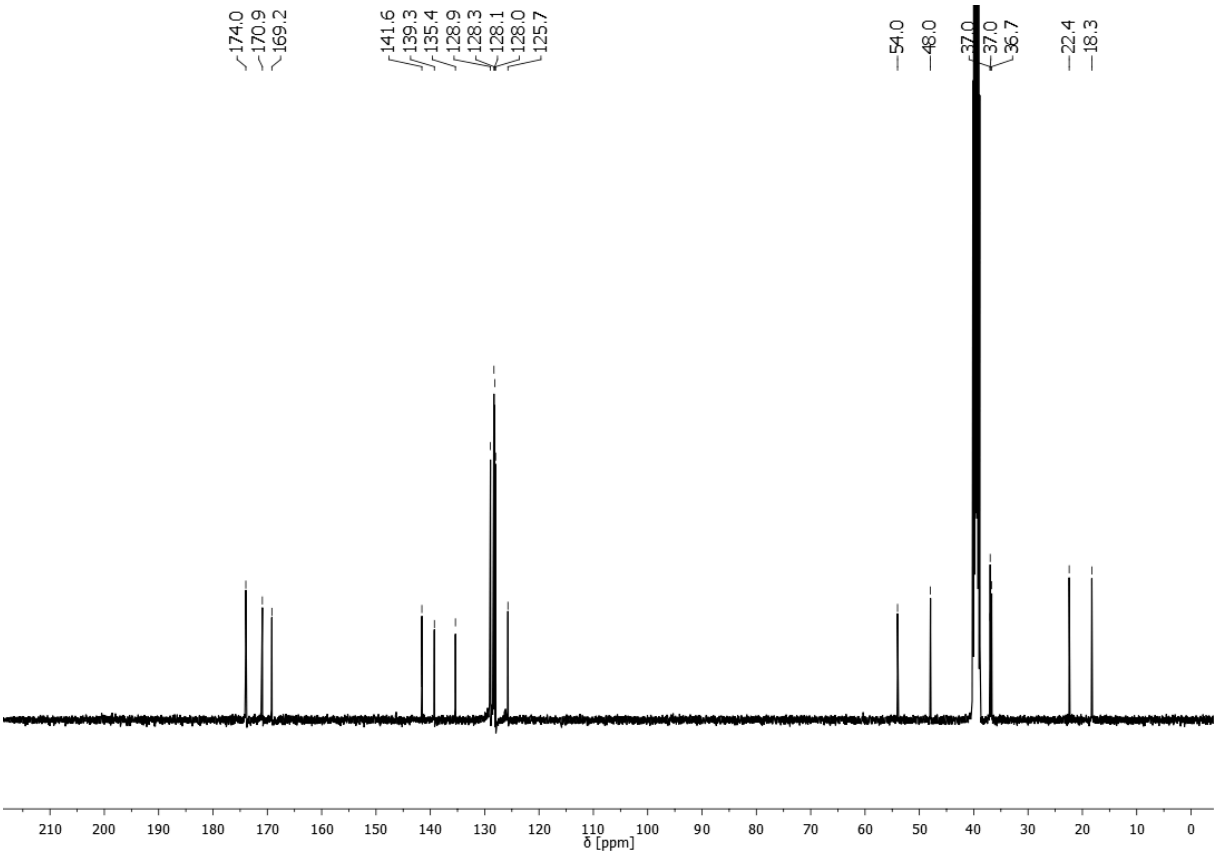
Purification by preparative HPLC provided the target material as a white amorphous solid. Yield: 28.2 mg (91%, crude, 71% purity), 12.1 mg (39%, purified). *rp*-HPLC (C-8): t_R = 20.6 min. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.04 (d, J = 8.2 Hz, 1H, NH_{Phe}), 7.98 (d, J = 7.5 Hz, 1H, NH_{Ala}), 7.32 – 7.03 (m, 10H, 9$H_{arom.}$, 1H_{NH2}), 7.04 (d, J = 52 Hz, 2H, NH_2), 4.56 – 4.37 (m, 1H, $H_{\alpha Phe}$), 4.19 (p, J = 7.1 Hz, 1H, $H_{\alpha Ala}$), 2.90 – 2.78 (m, 4H, 2×CH_2), 2.83 (ddd, J = 23.8, 13.9, 7.2 Hz, 2H, $H_{\beta Phe}$), 1.76 (s, 3H, CH_3), 1.21 (d, J = 7.1 Hz, 3H, $H_{\beta Ala}$). ^{13}C-NMR (101 MHz, DMSO-d_6): δ 174.0, 170.9, 169.2, 141.6, 139.3, 135.4, 128.9, 128.3, 128.2, 128.0, 125.7, 54.0, 48.0, 37.0, 37.0, 36.7, 22.4, 18.3. MS (ESI$^+$): m/z = 381.9 (calcd 382.2 for [M+H]$^+$). HR-MS (ESI$^+$): m/z = 382.2131 (calcd 382.2131 for $C_{22}H_{28}N_3O_3$).



HPLC chromatogram λ = 214 nm of peptide **4**.

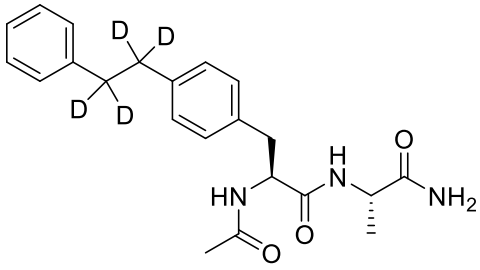


^{1}H NMR spectrum (400 MHz, DMSO-d_6) of peptide **4**.

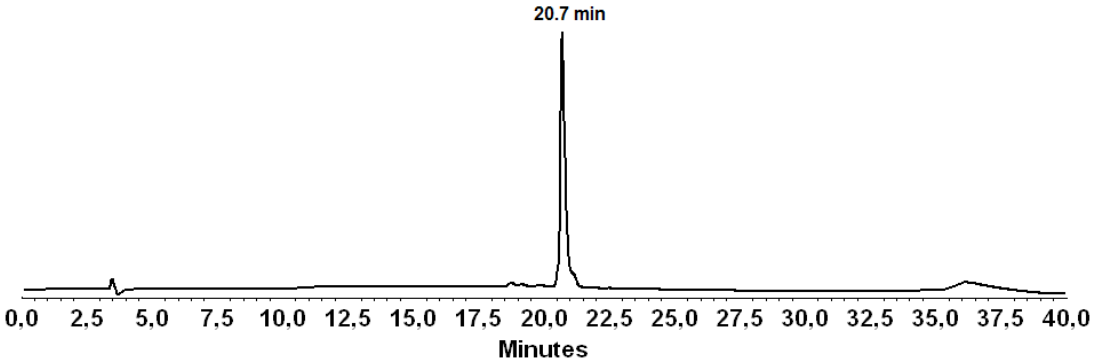


^{13}C NMR spectrum (101 MHz, DMSO-d_6) of peptide **4**.

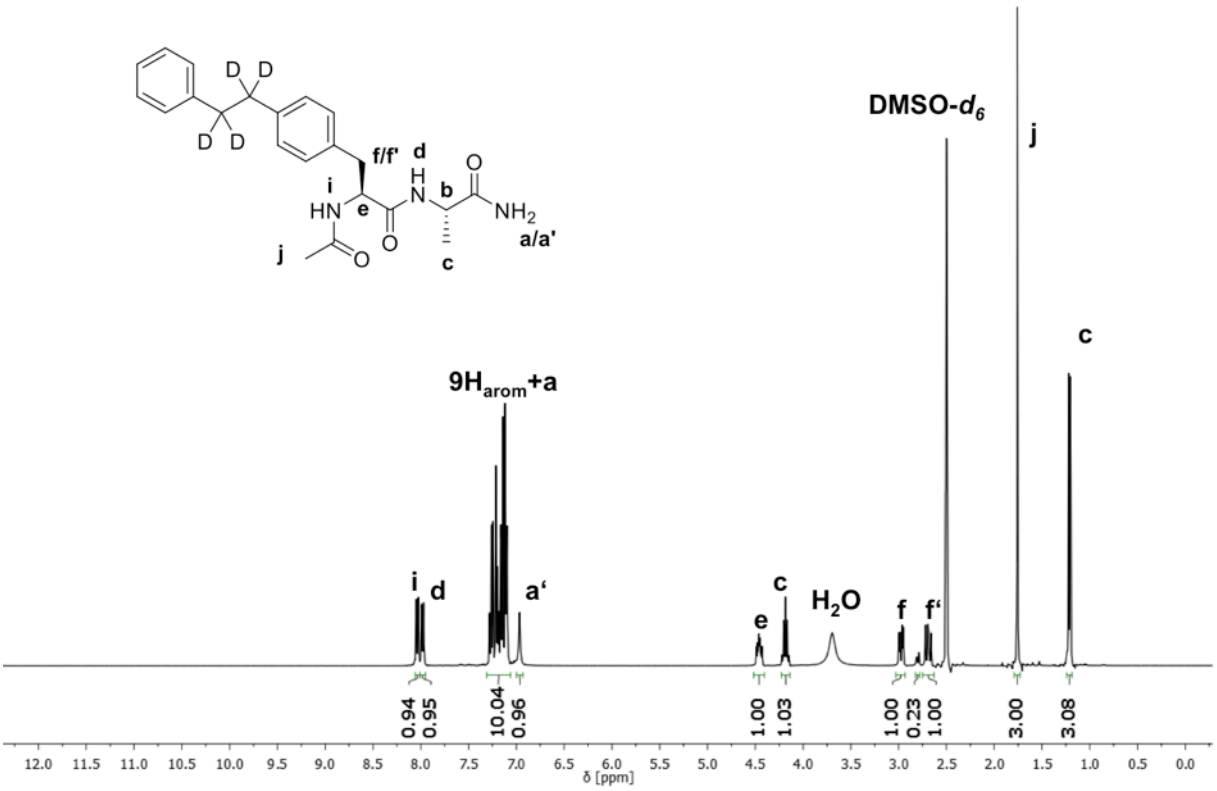
Ac-[4-(2-phenyl-$(CD_2)_2$-Phe]-Ala-NH_2 (8)



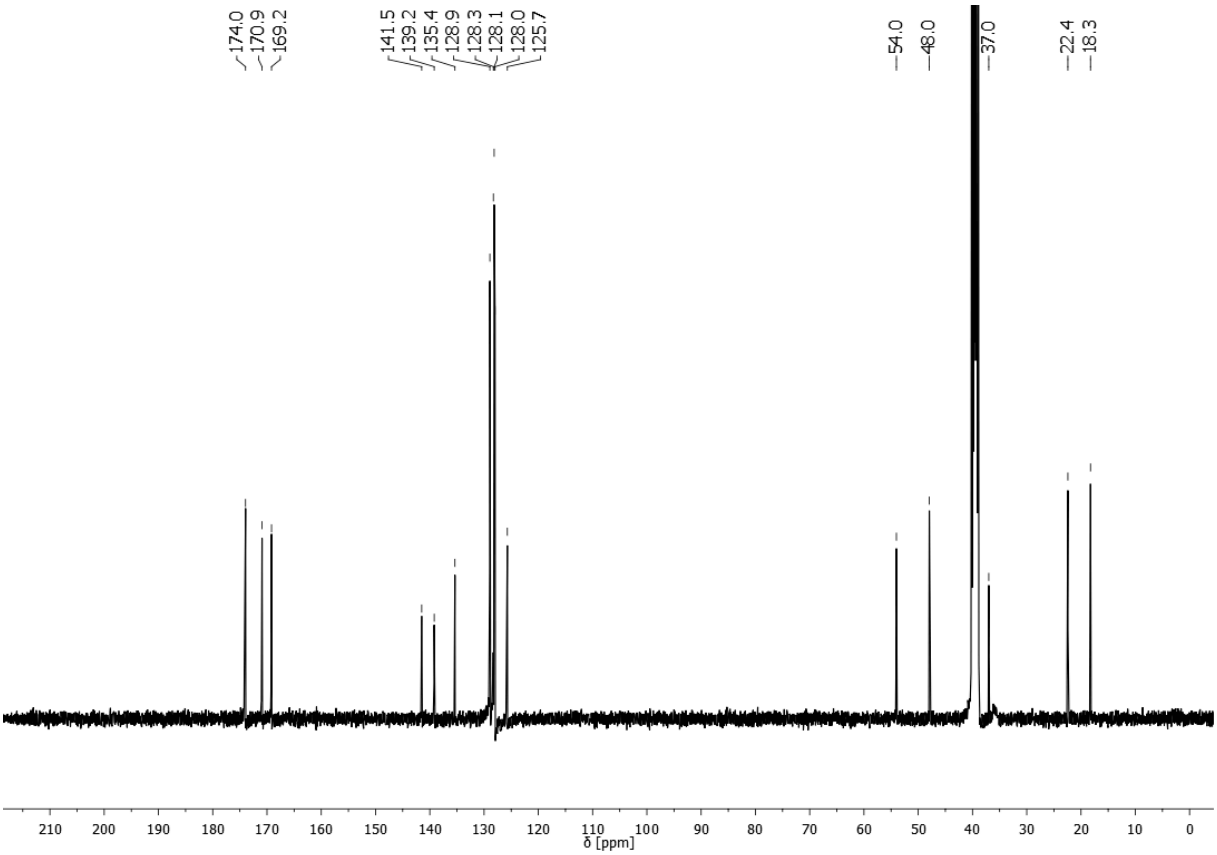
Purification by preparative HPLC provided the target peptide as a white amorphous solid. Yield: 27.5 mg (88%, crude, 70% purity), 9.5 mg (30%, purified). *rp*-HPLC (C-8): t_R = 20.7 min. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.04 (d, J = 8.2 Hz, 1H, NH_{Phe}), 7.98 (d, J = 7.5 Hz, 1H, NH_{Ala}), 7.31 – 7.06 (m, 10H, 9$H_{arom.}$, 1H_{NH2}), 7.04 (d, J = 52 Hz, 2H, NH_2), 4.54 – 4.39 (m, 1H, $H_{\alpha Phe}$), 4.18 (p, J = 7.1 Hz, 1H, $H_{\alpha Ala}$), 2.83 (ddd, J = 23.8, 13.9, 7.2 Hz, 2H, $H_{\beta Phe}$), 1.76 (s, 3H, CH_3), 1.21 (d, J = 7.1 Hz, 3H, $H_{\beta Ala}$). ^{13}C-NMR (101 MHz, DMSO-d_6): δ 174.0, 170.9, 169.2, 141.5, 139.2, 135.4, 128.9, 128.1, 128.0, 125.7, 54.0, 48.0, 37.0, 36.0 (HSQC), 22.4, 18.3. MS (ESI$^+$): m/z = 385.9 (calcd 386.2 for [M+H]$^+$). HR-MS (ESI$^+$): m/z = 386.2377 (calcd 386.2382 for $C_{22}H_{25}D_4N_3O_3$).



HPLC chromatogram λ = 214 nm of deuterated peptide **8**.

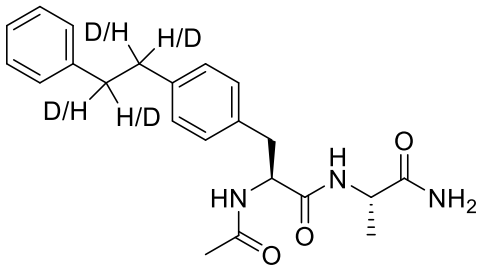


^{1}H NMR spectrum (400 MHz, DMSO-d_6) of deuterated peptide **8**.



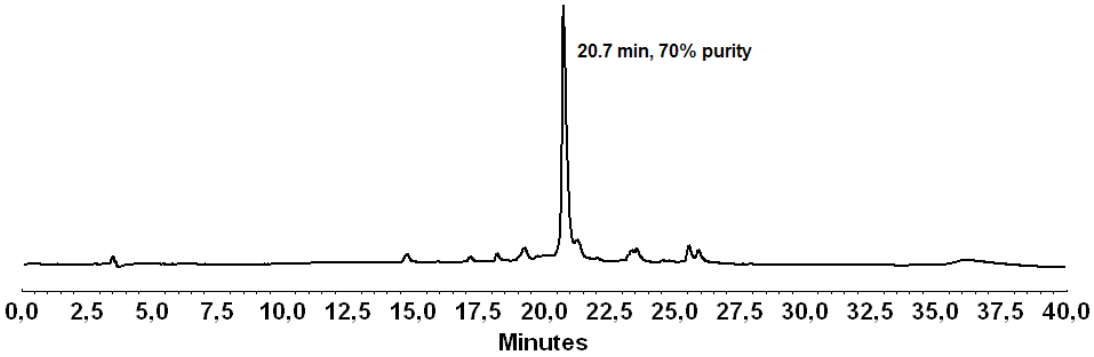
^{13}C NMR spectrum (101 MHz, DMSO-d_6) of deuterated peptide **8**.

Mixed Deuteration Experiments



Partially deuterated peptide 7

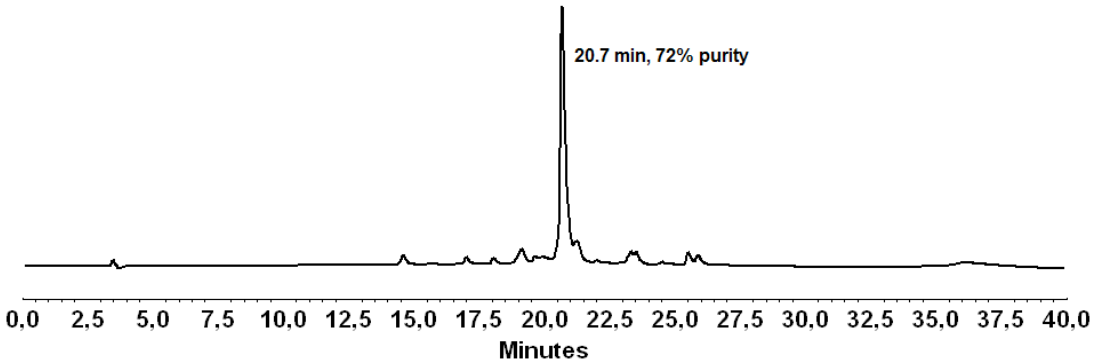
According to General Procedure A using *d*-TFA/D_2O/TES. The crude material was lyophilized three times from MeCN/H_2O (1:1, 15 mL) and a small aliquot was purified by *rp*-HPLC for MS analysis. Yield: 27.2 mg (87%, crude, 70% purity). *rp*-HPLC (C-8): t_R = 20.7 min.



HPLC chromatogram λ = 214 nm of partially deuterated peptide **7**.

Partially deuterated peptide 6

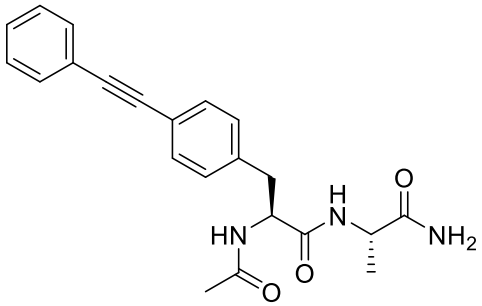
According to General Procedure A using TFA/H_2O/*d*-TES. The crude material was lyophilized three times from MeCN/H_2O (1:1, 15 mL) and a small aliquot was purified by *rp*-HPLC for MS analysis. Yield: 29.5 mg (95%, crude 72% purity). *rp*-HPLC (C-8): t_R = 20.7 min.



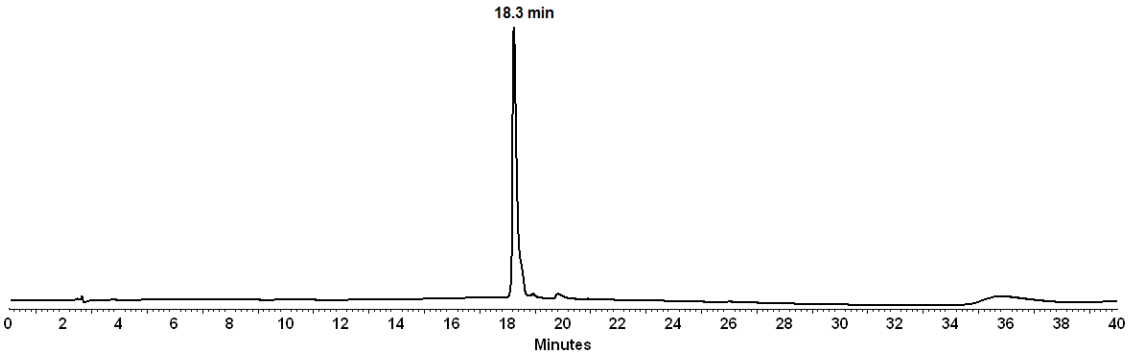
HPLC chromatogram λ = 214 nm of partially deuterated peptide **6**.

Ac-[4-(2-phenylethynyl)-Phe]-Ala-NH_2 (5a)

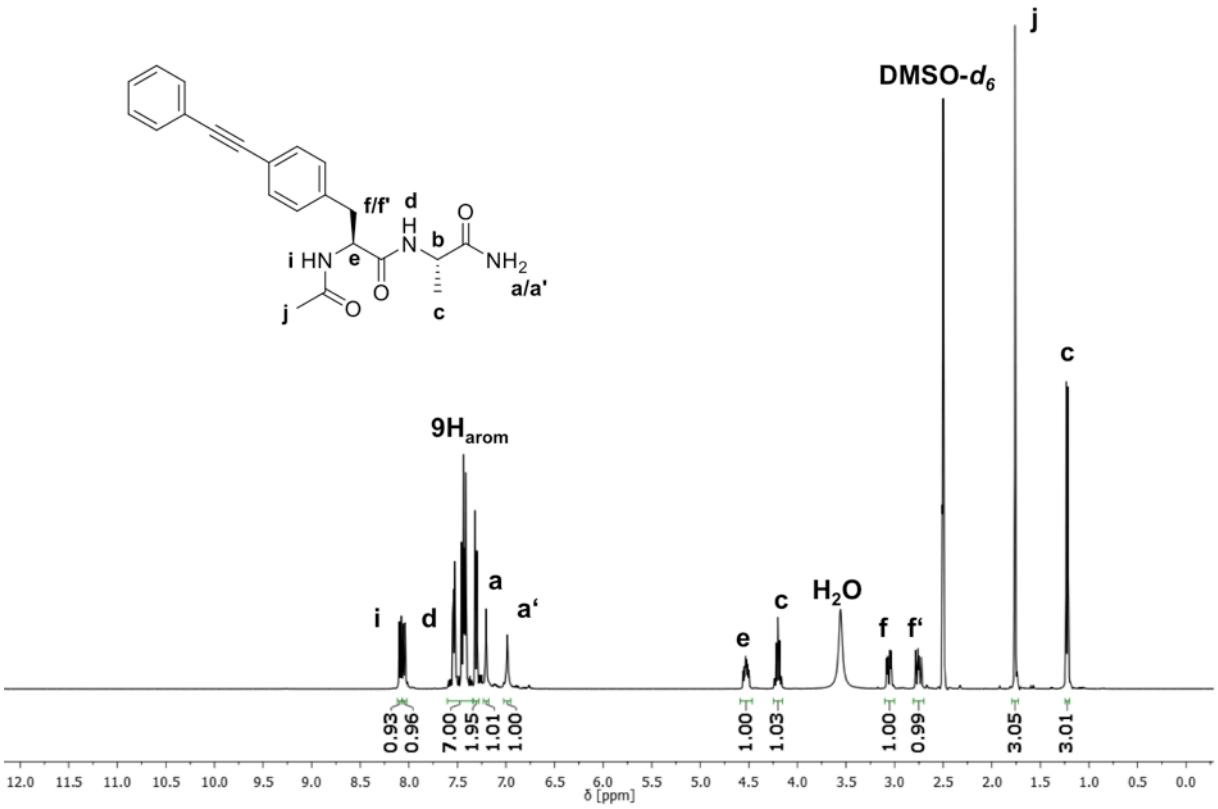
A solution of TFA/phenol (90:10, v/w, V_{tot} = 1 mL) was added to dry Ac-[4-(2-phenylethynyl)-Phe]-Ala-Rink resin **3a** (0.1 g, 54 µmol) and the suspension was agitated at room temperature for 60 min. The solution was separated by filtration and the resin was washed with TFA (2 × 1 mL × 1 min). The TFA fractions were combined and concentrated *in vacuo*. Addition of ice-cold Et_2O (25 mL) followed by centrifugation and two washing cycles with Et_2O (25 mL) yielded the crude material that was purified by preparative HPLC to yield the target compound as a white amorphous solid.



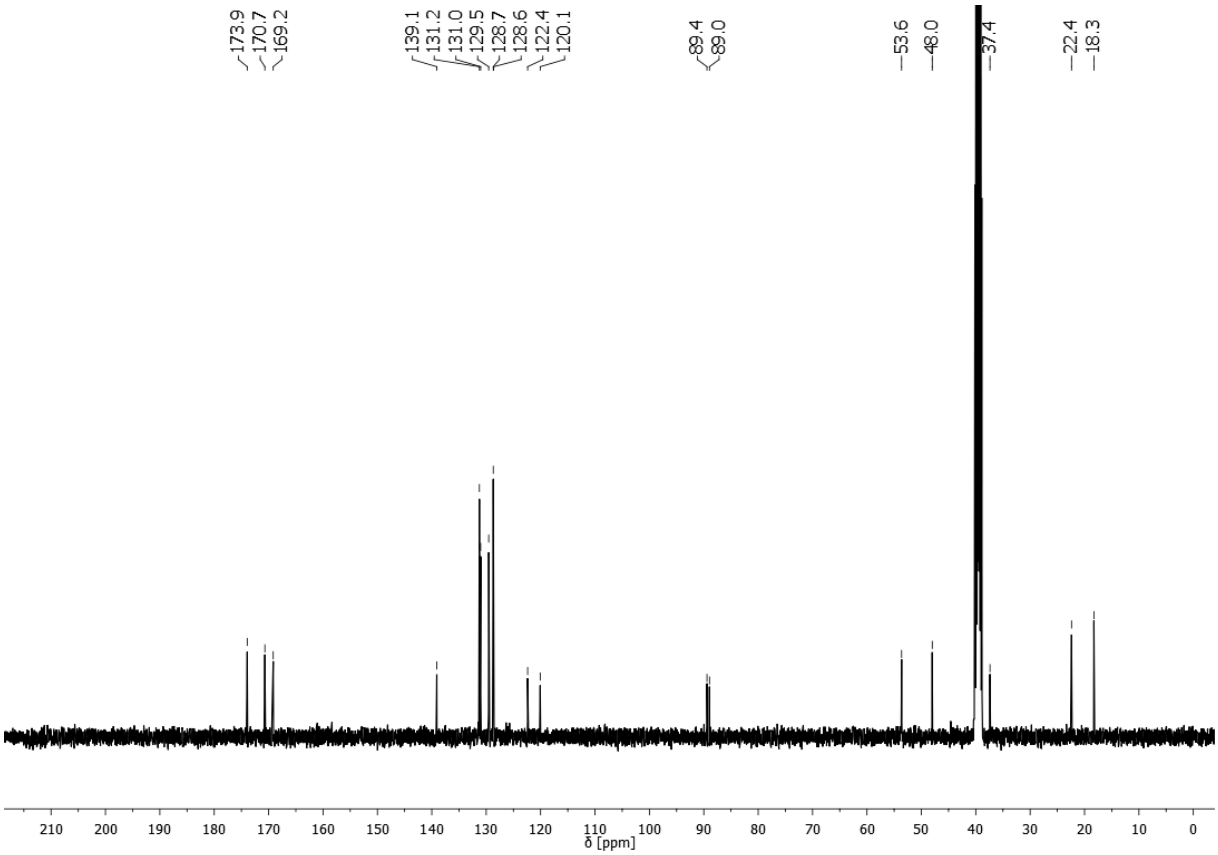
Yield: 7.4 mg (36%, purified). *rp*-HPLC (C-18): t_R = 18.3 min. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.09 (d, J = 8.4 Hz, 1H, NH_{Phe}), 8.05 (d, J = 7.6 Hz, 1H, NH_{Ala}), 7.60 – 7.34 (m, 7H, $H_{arom.}$), 7.31 (d, J = 8.2 Hz, 2H, $H_{arom.}$), 7.10 (d, J = 87.9 Hz, 2H, NH_2), 4.59 – 4.47 (m, 1H, $H_{\alpha Phe}$), 4.20 (p, J = 7.1 Hz, 1H, $H_{\alpha Ala}$), 2.91 (ddd, J = 23.8, 13.8, 7.2 Hz, 2H, $H_{\beta Phe}$), 1.76 (s, 3H, CH_3), 1.22 (d, J = 7.1 Hz, 3H, $H_{\beta Ala}$). ^{13}C-NMR (400 MHz, DMSO-d_6): δ 173.9, 170.7, 169.2, 139.1, 131.2, 131.0, 129.5, 128.7, 128.6, 122.4, 120.1, 89.4, 89.0, 53.6, 48.0, 37.4, 22.4, 18.3. MS (ESI$^+$): m/z = 377.9 (calcd 378.2 for $[M+H]^+$). HR-MS (ESI$^+$): m/z = 378.1819 (calcd 378.1818 for $C_{22}H_{23}N_3O_3$).



HPLC chromatogram λ = 214 nm of peptide **5a**.



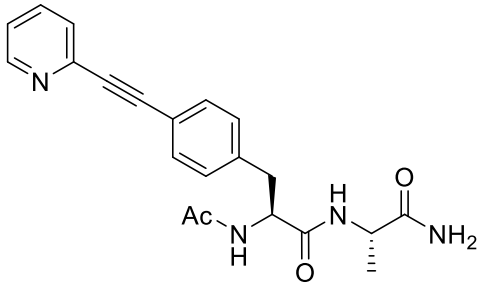
^{1}H NMR spectrum (400 MHz, DMSO-d_6) of peptide **5a**.



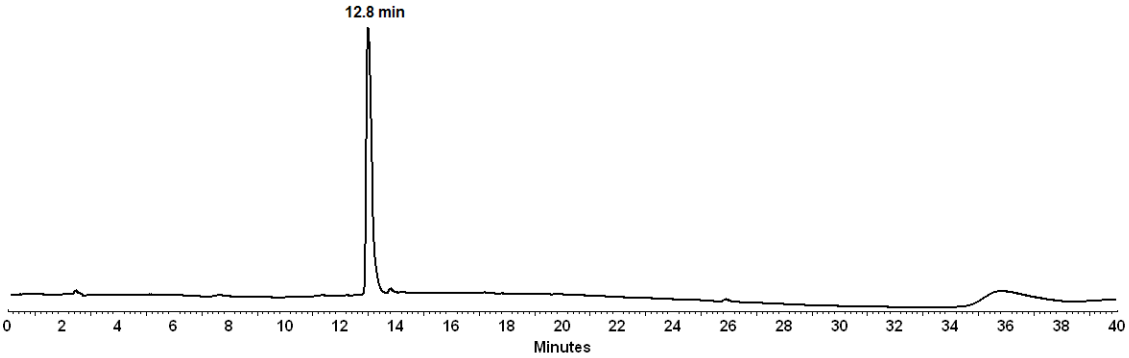
^{13}C NMR spectrum (101 MHz, DMSO-d_6) of peptide **5a**.

Ac-[4-(pyridin-2-ylethynyl)-Phe]-Ala-NH_2 (5b)

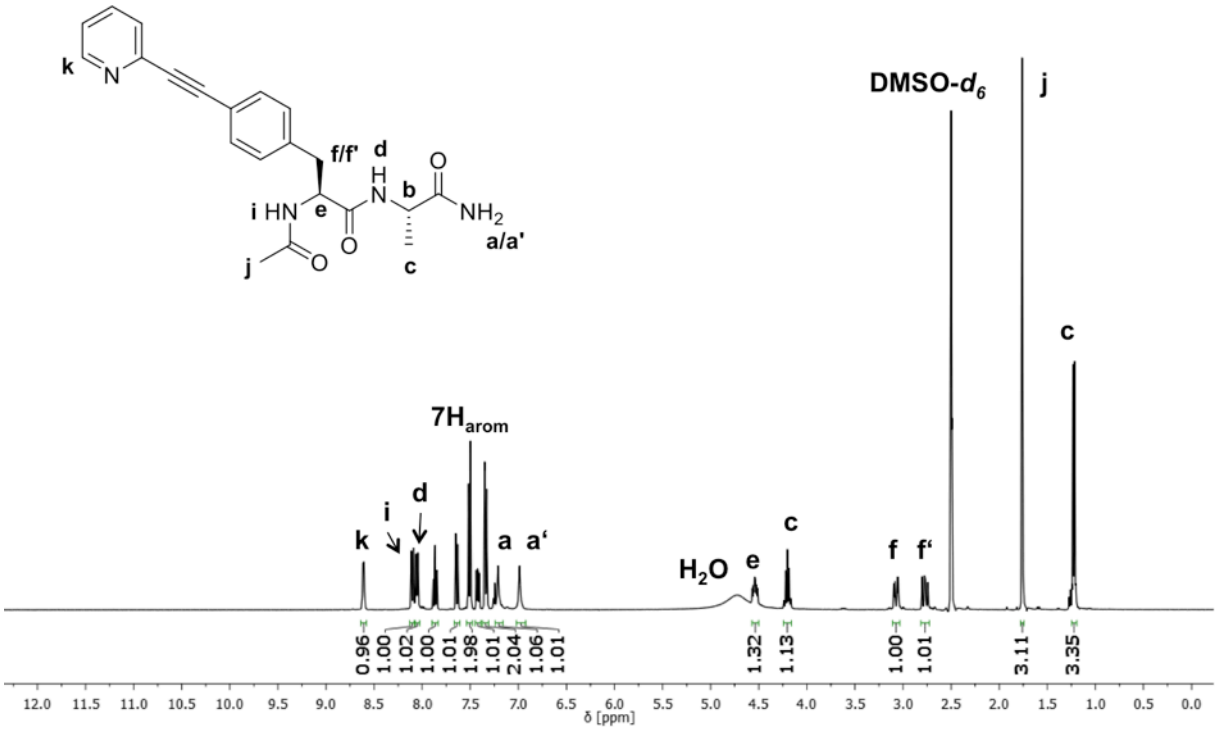
In a one-neck Schlenk flask, a suspension of Ac-[(4-I)-Phe]-Ala-Rink solid support **3b** (0.2 g, 0.108 mmol), 2-ethynylpyridine (16.4 µL, 0.162 mmol) and D*i*PEA (73.3 µL, 0.432 mmol) was prepared and degassed. Then, $(PPh_3)_2PdCl_2$ (22.7 mg, 0.032 mmol) and CuI (12.3 mg, 0.065 mmol) were added. The suspension was stirred at room temperature overnight. The resin was isolated by filtration and washed with DCM (5 × 10 mL × 2 min), DMF (5 × 10 mL × 2 min), DCM (4 × 10 mL × 2 min) and Et_2O (3 × 10 mL × 2 min) and was dried *in vacuo*. Ac-[4-(pyridine-2-ylethynyl)-Phe]-Ala-Rink solid support (0.1 g, 54 µmol) was treated with a mixture of TFA/phenol (9:1, v/w, V_{tot} = 1 mL) for 60 min. The resin was isolated by filtration and was washed with TFA (2 × 0.5 mL × 1 min). Combined TFA solutions were concentrated *in vacuo* and Et_2O (20 mL) was added to the residual material. The formed precipitate was isolated by centrifugation and was washed with Et_2O (2 × 20 mL). Preparative HPLC yielded the target compound as a white amorphous solid.



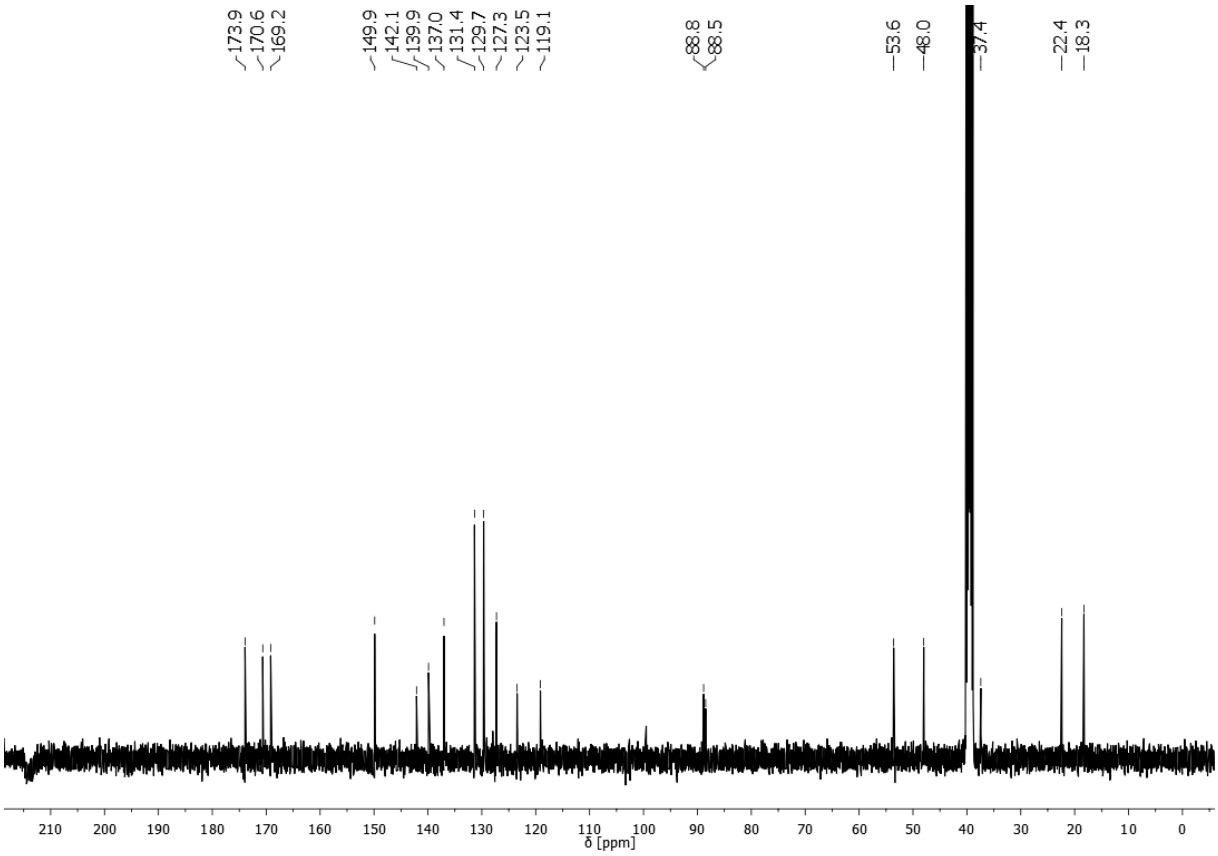
Yield: 9.4 mg (35%). *rp*-HPLC (C-18): t_R = 12.8 min. ^{1}H NMR (400 MHz, DMSO) δ 8.61 (d, *J* = 4.7 Hz, 1H, $H_{arom.}$), 8.10 (d, *J* = 8.4 Hz, 1H, NH_{Phe}), 8.05 (d, *J* = 7.5 Hz, 1H, NH_{Ala}), 7.87 (td, *J* = 7.7, 1.8 Hz, 1H, $H_{arom.}$), 7.64 (d, *J* = 7.8 Hz, 1H, $H_{arom.}$), 7.51 (d, *J* = 8.1 Hz, 2H, $H_{arom.}$), 7.42 (ddd, *J* = 7.6, 4.9, 1.1 Hz, 1H, $H_{arom.}$), 7.34 (d, *J* = 8.2 Hz, 2H, $H_{arom.}$), 7.10 (d, *J* = 89.6 Hz, 2H, NH_2), 4.59–4.48 (m, 1H, $H_{αPhe}$), 4.20 (p, *J* = 7.1 Hz, 1H, $H_{αAla}$), 2.92 (ddd, *J* = 23.8, 13.8, 7.2 Hz, 2H, $H_{βPhe}$), 1.76 (s, 3H, CH_3), 1.22 (d, *J* = 7.1 Hz, 3H, $H_{βAla}$). ^{13}C-NMR (101 MHz, DMSO-d_6): *δ* 173.9, 170.6, 169.2, 149.9, 142.1, 139.9, 137.0, 131.1, 129.7, 127.3, 123.5, 119.1, 88.8, 88.5, 53.6, 48.0, 37.4, 22.4, 18.3. MS (ESI$^+$): *m/z* = 378.9 (calcd 379.2 for [M+H]$^+$). HR-MS (ESI$^+$): *m/z* = 379.1771 (calcd 379.1770 for $C_{21}H_{23}N_4O_3$).



HPLC chromatogram λ = 214 nm of peptide **5b**.



^{1}H NMR spectrum (400 MHz, DMSO-d_6) of peptide **5b**.

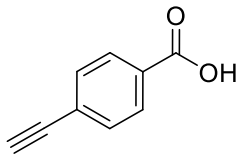


^{13}C NMR spectrum (400 MHz, DMSO-d_6) of peptide **5b**.

Bisarylethyne-bridged peptides

4-Ethynylbenzoic Acid

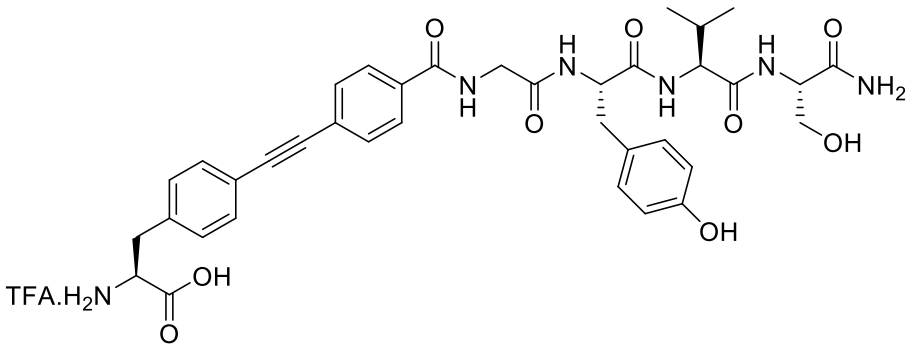
Prepared according to a literature procedure.[1]



Yield: 181.0 mg (62%, 2 steps). R_f (SiO_2, PE:EtOAc – 1:1, v/v) = 0.1. ^{1}H-NMR (DMSO-d_6, 200 MHz): δ 7.93 (d, J = 8.2 Hz, 2H, $H_{arom.}$), 7.59 (d, J = 8.2 Hz, 2H, $H_{arom.}$), 4.43 (s, 1H, C≡C*H*). ^{13}C-NMR (DMSO-d_6, 53 MHz): δ 166.7, 131.9, 130.9, 129.5, 126.0, 83.6, 82.2. MS (EI$^+$): *m/z* = 146.0 (calcd 146.0 for [M]$^+$).

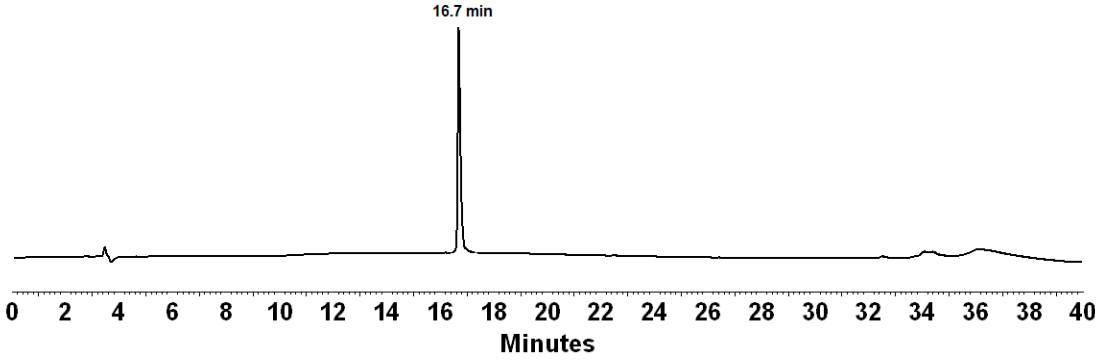
Conjugated 4-iodophenylalanine (10)

H-Gly-Tyr(^{t}Bu)-Val-Ser(^{t}Bu)-Rink-PS **9** (0.1 g, 0.053 mmol) was prepared as described above. A solution of 4-ethynylbenzoic acid (15.5 mg, 0.106 mmol), PyBOP (53.8 mg, 0.103 mmol) and D*i*PEA (18.0 µL, 0.106 mmol) in DMF (0.5 mL) was prepared and added to the resin-bound peptide. The suspension was agitated for 4 h, the resin was isolated by filtration and was washed with DMF (4 × 2 mL × 2 min), DCM (3 × 2 mL × 2 min) and Et_2O (3 × 2 mL × 2 min) and was dried *in vacuo*. The acetylene derivatized peptide resin was placed in a one-neck Schlenk flask and a solution of Fmoc-[(4-I)-Phe]-OH (31.6 mg, 0.06 mmol) and D*i*PEA (37.2 µL, 0.22 mmol) in DMF (0.5 mL) was added and the mixture was degassed thoroughly. Then, $(PPh_3)_2PdCl_2$ (11.6 mg, 0.017 mmol) and CuI (6.3 mg, 0.033 mmol) were added and the reaction mixture was stirred at room temperature overnight. The resin was isolated by filtration and washed with DCM (5 × 3 mL × 2 min), DMF (5 × 3 mL × 2 min), DCM (4 × 3 mL × 2 min) and was treated with 20% piperidine in NMP (2 × 2 mL × 10 min) for Fmoc deprotection of the phenylalanine moiety. Subsequently, the solid support was washed with DMF (5 × 3 mL × 2 min), DCM (4 × 3 mL × 2 min) and Et_2O (3 × 3 mL × 2 min) and was dried *in vacuo*. The material was treated with a mixture of TFA/phenol (9:1, v/w, V_{tot} = 1 mL) for 60 min. The resin was isolated by filtration and was washed with TFA (2 × 0.5 mL × 1 min). Combined TFA solutions were concentrated *in vacuo* and Et_2O (25 mL) was added to the residual material. The formed precipitate was isolated by centrifugation and was washed with Et_2O (2 × 25 mL). Preparative HPLC yielded the target compound as a white amorphous solid.

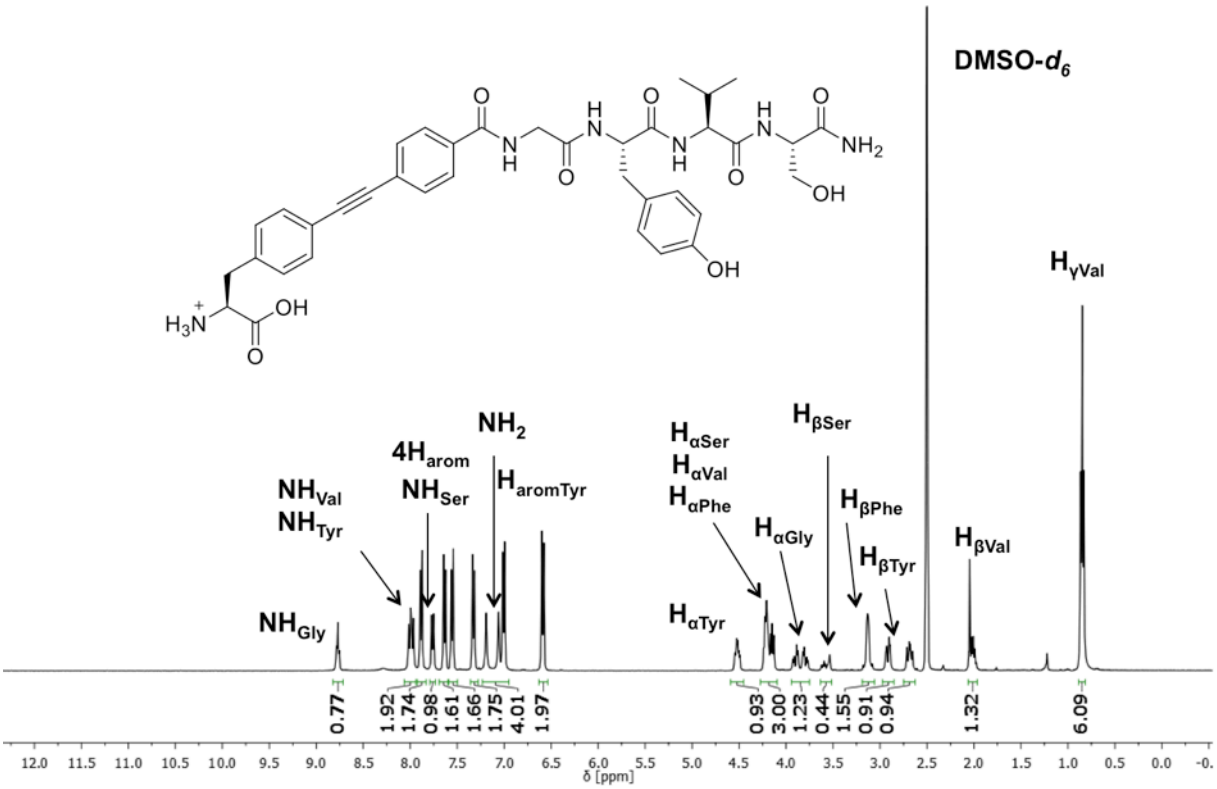


Yield: 10.8 mg (25%, 13 steps). *rp*-HPLC (C-18): t_R = 16.7 min. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.77 (t, J = 5.8 Hz, 1H, NH_{Gly}), 8.05 – 7.93 (m, 2H, NH_{Tyr}, NH_{Val}), 7.88 (d, J = 8.4 Hz,

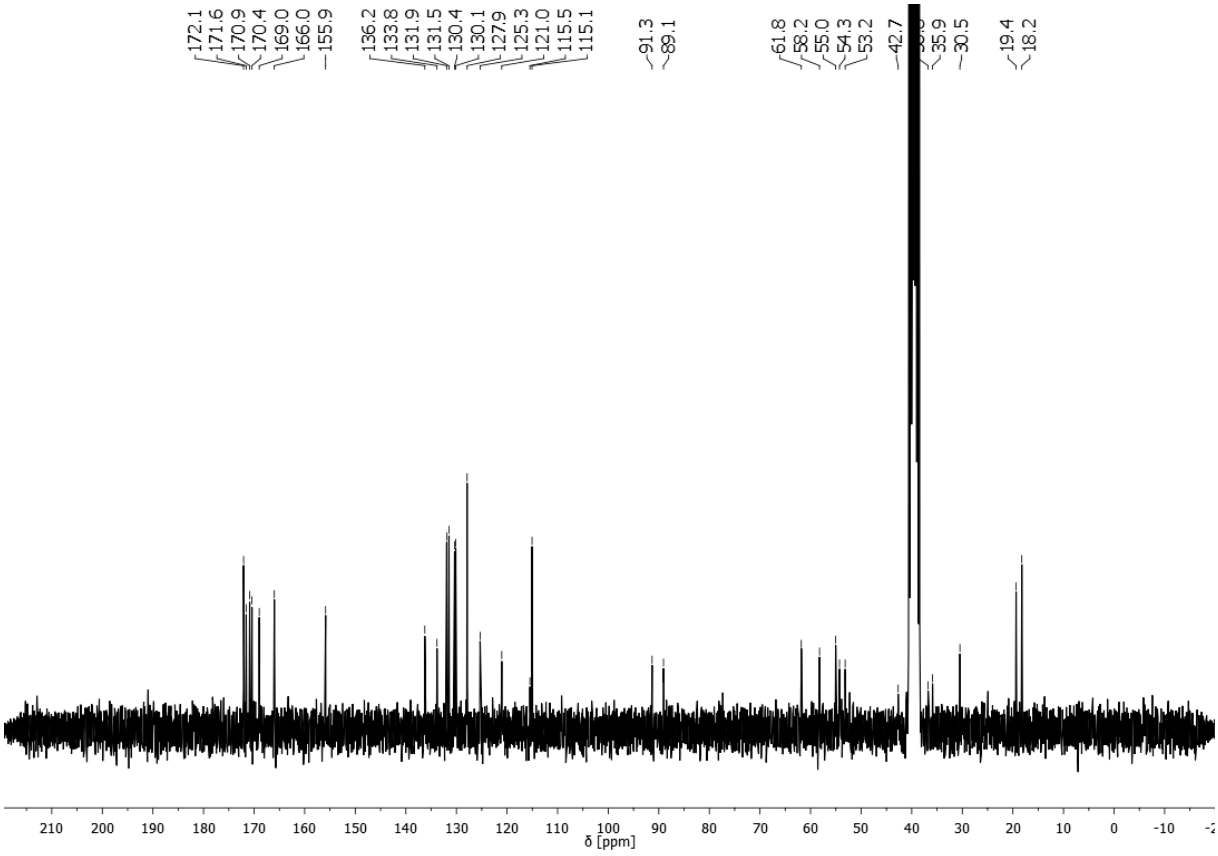
2H, $H_{arom.}$), 7.76 (d, J = 7.8 Hz, 1H, NH_{Ser}), 7.63 (d, J = 8.4 Hz, 2H, $H_{arom.}$), 7.55 (d, J = 8.2 Hz, 2H, $H_{arom.}$), 7.33 (d, J = 8.3 Hz, 2H, $H_{arom.}$), 7.13 (d, J = 53.3 Hz, 2H, NH_2), 7.00 (d, J = 8.5 Hz, 2H, $H_{arom.Tyr}$), 6.59 (d, J = 8.4 Hz, 2H, $H_{arom.}$), 4.58 – 4.45 (m, 1H, $H_{\alpha Tyr}$), 4.28 – 4.10 (m, 3H, $H_{\alpha Ser}$, $H_{\alpha Val}$, $H_{\alpha Phe}$), 3.96 – 3.73 (m, 2H, $H_{\alpha Gly}$), 3.65 – 3.50 (m, $H_{\beta Ser}$), 3.21 – 3.05 (m, $H_{\beta Phe}$), 2.92 (dd, J = 13.8, 3.6 Hz, 1H, $H_{\beta Tyr}$), 2.68 (dd, J = 13.9, 9.4 Hz, 1H, $H_{\beta Tyr}$), 2.07 – 1.95 (m, 1H, $H_{\beta Val}$), 0.85 (t, J = 6.9 Hz, 6H, $H_{\gamma Val}$). ^{13}C NMR (63 MHz, DMSO-d_6): δ 172.1, 171.6, 170.9, 170.4, 169.0, 166.0, 136.2, 133.8, 131.9, 131.5, 130.4, 130.1, 127.9, 125.3, 121.0, 115.5, 115.1, 91.3, 89.1, 61.8, 58.2, 54.3, 53.2, 42.7 (HSQC), 36.8 (HSQC), 35.9 (HSQC), 30.5; 19.4, 18.5 (rotamers, $C_{\gamma Val}$). MS (ESI$^+$): m/z = 715.1 (calcd 715.3 for [M+H]$^+$). HR-MS (ESI$^+$): m/z = 715.3087 (calcd 715.3091 for $C_{37}H_{43}N_6O_9$).



HPLC chromatogram λ = 214 nm of bisarylethyne-bridged peptide **10**.



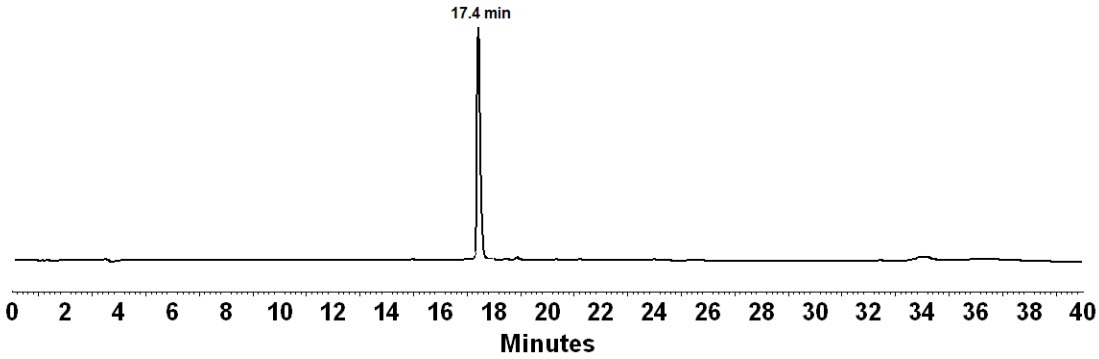
^{1}H NMR spectrum (400 MHz, WATERGATE DMSO-d_6) of bisarylethyne-bridged peptide **10**.



^{13}C NMR spectrum (400 MHz, DMSO-d_6) of bisarylethyne-bridged peptide **10**.

Conjugated Ac-[(4-I)-Phe]-Ser-NH$_2$ (12)

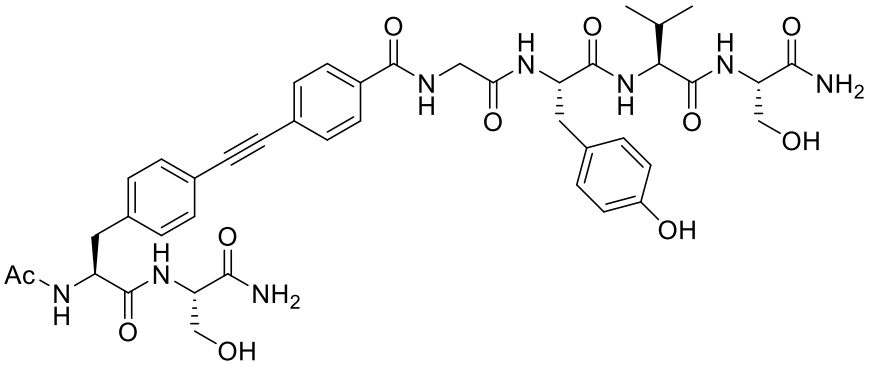
Dry Ac-[(4-I)-Phe]-Ser(^{t}Bu)-Rink-PS (0.2 g, 0.11 mmol) was prepared *via* the method that was described for the preparation of peptide **1**. Cleavage was performed with a mixture of TFA/phenol (9:1, v/w, V_{tot} = 2 mL) at room temperature for 1 h. The resin was isolated by filtration and was washed with TFA (2 × 1 mL × 1 min). After concentration of the combined TFA solutions and subsequent precipitation and washing with Et_2O (3 × 25 mL), the peptide material **11** (25.9 mg, 58%) was obtained as a white amorphous solid with high purity; *rp*-HPLC (C-18): t_R = 17.4 min. MS (ESI$^+$): *m/z* = 419.7 (calcd 420.2 for [M+H]$^+$).



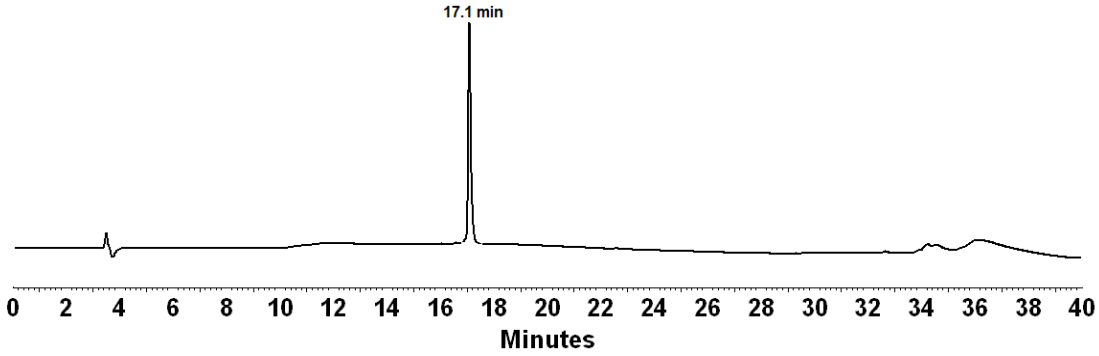
HPLC chromatogram λ = 214 nm of Ac-(4-I)Phe-Ser-NH$_2$ **11**.

H-Gly-Tyr(^{t}Bu)-Val-Ser(^{t}Bu)-Rink-PS **9** (0.1 g, 0.053 mmol) was prepared as described above. A solution of 4-ethynylbenzoic acid (15.5 mg, 0.106 mmol), PyBOP (53.8 mg, 0.103 mmol) and D*i*PEA (18.0 µL, 0.106 mmol) in DMF (0.5 mL) was prepared and added to the

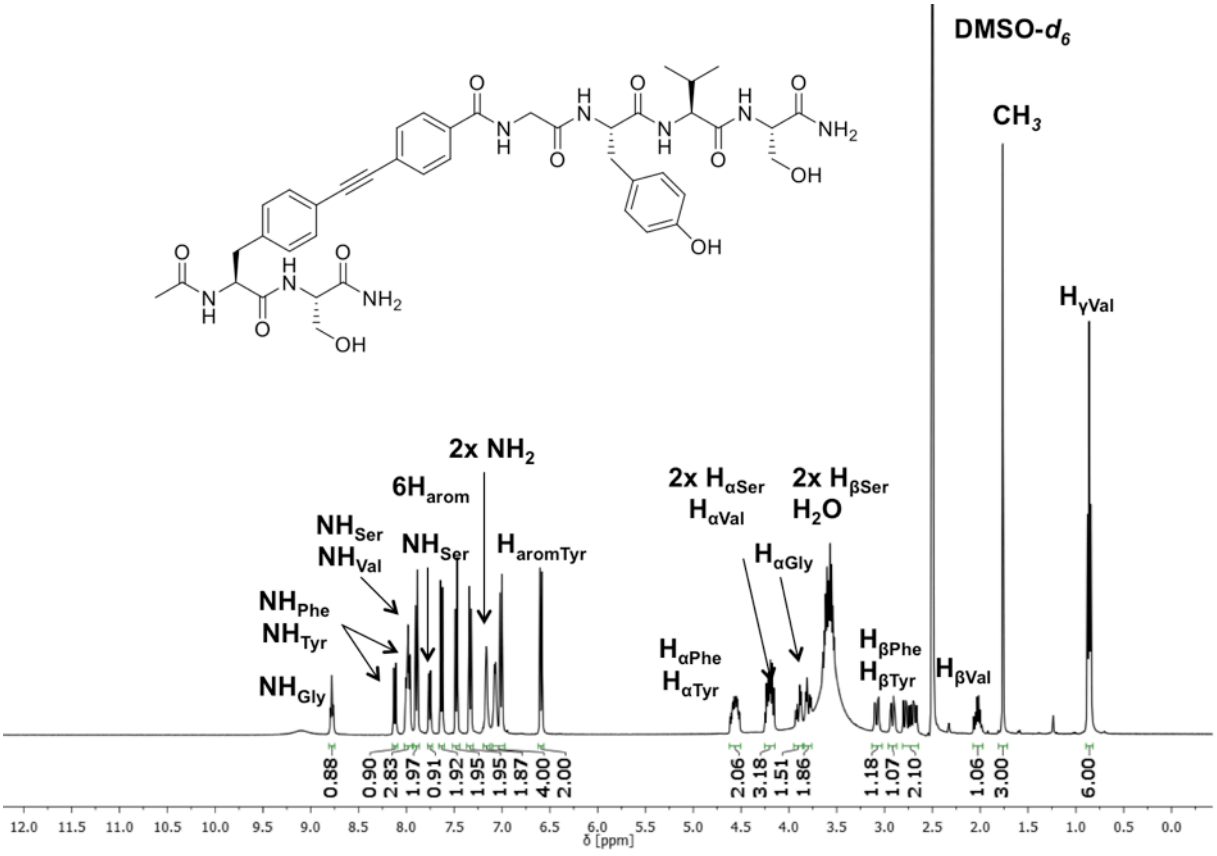
resin-bound peptide. The suspension was agitated for 4 h, the resin was isolated by filtration and was washed with DMF (4 × 2 mL × 2 min), DCM (3 × 2 mL × 2 min) and Et_2O (3 × 2 mL × 2 min) and was dried *in vacuo*. In a one-neck Schlenk flask, the obtained resin was combined with Ac-[(4-I)-Phe]-Ser-NH_2 (24.5 mg, 0.058 mmol), D*i*PEA (0.1 mL, 0.58 mmol) and DMF (1.5 mL) and the mixture was degassed. Subsequently, $(PPh_3)_2PdCl_2$ (11.2 mg, 0.016 mmol) and CuI (6.1 mg, 0.032 mmol) were added and the mixture was stirred at room temperature overnight. The resin was isolated by filtration and washed with DCM (5 × 3 mL × 2 min), DMF (5 × 3 mL × 2 min), DCM (4 × 3 mL × 2 min) and Et_2O (3 × 3 mL × 2 min) and was dried *in vacuo*. The material was treated with a mixture of TFA/phenol (9:1, v/w, V_{tot} = 1 mL) for 60 min. The resin was isolated by filtration and was washed with TFA (2 × 0.5 mL × 1 min). Combined TFA solutions were concentrated *in vacuo* and Et_2O (25 mL) was added to the residual material. The formed precipitate was isolated by centrifugation and was washed with Et_2O (2 × 25 mL). Preparative HPLC yielded the target compound as a white amorphous solid.



Yield: 10.0 mg (22%, 12 steps). *rp*-HPLC (C-18): t_R = 17.1 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.78 (t, *J* = 5.9 Hz, 1H, NH_{Gly}), 8.12 (d, *J* = 8.4 Hz, 1H, N$H_{Tyr/Phe}$), 8.04 – 7.94 (m, 3H, N$H_{Tyr/Phe}$, NH_{Val}, NH_{Ser1}), 7.89 (d, *J* = 8.4 Hz, 2H, $H_{arom.}$), 7.76 (d, *J* = 7.8 Hz, 1H, NH_{Ser2}), 7.63 (d, *J* = 8.4 Hz, 2H, $H_{arom.}$), 7.48 (d, *J* = 8.1 Hz, 2H, $H_{arom.}$), 7.33 (d, *J* = 8.3 Hz, 2H, $H_{arom.}$), 7.19 – 7.04 (m, 4H, 2×NH_2), 7.01 (d, *J* = 8.5 Hz, 2H, $H_{arom.Tyr}$), 6.59 (d, *J* = 8.4 Hz, 2H, $H_{arom.}$), 4.67 – 4.47 (m, 2H, $H_{\alpha Tyr,}$ $H_{\alpha Phe}$), 4.28 – 4.13 (m, 3H, 2×$H_{\alpha Ser}$, $H_{\alpha Val}$), 3.98 – 3.75 (m, 2H, $H_{\alpha Gly}$), 3.71 – 3.45 (m, $H_{\beta Ser}$), 3.08 (dd, *J* = 13.9, 4.2 Hz, 1H, $H_{\beta Tyr/Phe}$), 2.92 (dd, *J* = 13.9, 3.7 Hz, 1H, $H_{\beta Tyr/Phe}$), 2.83 – 2.64 (m, 2H, $H_{\beta Tyr/Phe}$), 2.08 – 1.95 (m, 1H, $H_{\beta Val}$), 1.76 (s, 3H, CH_3), 0.86 (t, *J* = 7.0 Hz, 6H, $H_{\gamma Val}$). ^{13}C NMR (extracted from HMBC experiment, DMSO-d_6): *δ* 171.8, 171.6, 171.1, 170.6, 169.1, 168.4, 165.6, 165.4, 155.7, 139.4, 133.5, 131.0, 130.3, 130.2, 129.4, 127.6, 127.3, 125.1, 119.7, 114.7, 91.4, 88.4, 61.6, 57.9, 55.1, 54.9, 54.3, 53.8, 53.3, 37.1, 36.5, 30.3, 30.2, 22.5, 18.5. MS (ESI$^+$): *m/z* = 843.1 (calcd 843.4 for $[M+H]^+$). HR-MS (ESI$^+$): *m/z* = 843.3676 (calcd 843.3677 for $C_{42}H_{51}N_8O_{11}$).



HPLC chromatogram λ = 214 nm of bisarylethyne-bridged peptide **12**.



^{1}H NMR spectrum (400 MHz, DMSO-d_6) of bisarylethyne-bridged peptide **12**.

Reference

(1) Jones, L. F.; Cochrane, M. E.; Koivisto, B. D.; Leigh, D. A.; Perlepes, S. P.; Wernsdorfer, W.; Brechin, E. K. *Inorganica Chimica Acta* **2008**, *361*, 3420.